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Research Article

Bacterial Isolates and Antimicrobial Susceptibility Profiles in Wound Swabs from Central Polokwane NHLS, in Limpopo Province, South Africa

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ABSTRACT

Bacteria are a major cause of wound infections with associated emergence of drug resistance to commonly used antimicrobials. This situation necessitates proper identification of the wound microbiota and their respective antimicrobial susceptibility profiles. A total of 88 wound swab specimens were collected and cultured by standard microbiological methods. The antibiotic susceptibility test of the bacterial isolates was performed by Kirby-Bauer disk diffusion method. Seven different species of bacteria were isolated. The most common organism isolated was *Staphylococcus aureus* (29%) followed by *Staphylococcus epidermidis* (15%), lactose fermenting coliforms (15%), *Pseudomonas* species (11%), *Klebsiella* species (7%) as well as *Escherichia coli* (3%) and *Streptococcus* group D (3%). Only 1% of *Staphylococcus saprophyticus* was isolated. All the positive samples were monomicrobial. Fourteen (14) samples showed no growth. The majority of the isolates were resistant to almost all the antimicrobials tested. *Streptococcus* group D was sensitive to mupirocin and oxacillin while *Escherichia coli* exhibited sensitivity for colistin sulphate and gentamicin. A high rate of multiple antibiotic resistant isolates was observed in both gram-positive and -negative bacteria. The results are presumptive of the likelihood of a changing resistant profile among the specimen tested. That might be attributable to various factors and warrants further investigation.

Keywords: Wound infections, bacteriology, susceptibility patterns, multi-drug resistance

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INTRODUCTION

Wound infections had been regarded as the most common nosocomial infection and present a major challenge to patients, health care staff and the general health care system in terms of costs and management (Sienkiewicz et al., 2014). The majority of these infections present in a number of clinical conditions like non-healing and burn wounds, injuries due to trauma, existing chronic infections like diabetic foot and pressure ulcers as well as post-operative and surgical wounds (Collier, 2004; Benwan et al., 2012; Braga et al., 2013; Aisha et al., 2013).

When the various clinical predicaments are presented, the skin loses its function as a first line barrier to infections acquired in the hospital or patient's own endogenous and exogenous flora (Nagoba et al., 2010; Aftab et al., 2014). Infection of wound is the successful invasion, proliferation by one or more species of microorganisms anywhere within the body's health tissues and sometimes resulting in pus

formation (Mama et al., 2014). The bacterial agents responsible for wound infections are derived from Gram-positive pathogens like *Staphylococcus aureus*, and *Streptococcus pyogenes* as well as Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Sienkiewicz et al., 2014; Cardona et al., 2016).

A number of factors expose patients to infections. In addition to the loss of skin due to trauma, extended hospital stay increases chances of acquiring infections in health care setups, routine use of invasive procedures and an immunosuppressive effect of trauma due to burn injury (Ashariak et al., 2004; Ndeegut et al., 2018). Moreover, wound infections with multiple micro-organisms (polymicrobial) as well as an added problem with drug resistance impacts on drug policy formulation by health care staff (Roberts et al., 2008; Das and Horton, 2016). The current spread of multi-drug resistant bacterial pathogens has added a new dimension to the problem of wound infections (Alharbi

and Zayed, 2014). This is particularly worse in resource-poor countries where sales of antibiotics are poorly controlled (Serena, 2014).

The objectives of this paper were to isolate and identify bacterial species in wound swabs from Central Polokwane NHLS (Limpopo Province, South Africa), as well as determining the antimicrobial susceptibility patterns of the isolated micro-organisms.

MATERIALS AND METHODS

Sample collection and microbial identification.

Clinical wound swabs (88) were collected from the Central Polokwane NHLS. They were cultured on blood, chocolate and McConkey agar plates. The blood and McConkey agar plates were incubated aerobically at 37°C for 24-48 h, while chocolate agar plate was incubated in CO₂ at 37°C 24-48 h. Colonies were inoculated on nutrient agar slants. The slants were incubated at 37° for 18-24h before storage in the refrigerator at 4°C pending biochemical analysis.

Identification of bacterial pathogens.

Pure cultures were characterized using morphological appearances on selective and differential media, Gram stain appearances and biochemical tests according to standard techniques (Wayne, 2005).

Antibiotics.

The six antimicrobials selected for Gam-positive bacteria were linezolid (30µg), mupirocin (5µg), oxacillin (1µg), synergid (15µg), teicoplanin (30µg) and vancomycin (30µg). Eight antibiotics were selected for Gram-negative bacteria; ampicillin (10µg), tetracycline (25µg), colistin sulphate (25µg), cephalothin (5µg), cotrimoxazole (25µg), gentamycin (10µg), streptomycin (10µg) and sulphatriad (200µg).

Antimicrobial susceptibility testing.

Antibiotic susceptibility determination was conducted using Kirby-Bauer disc diffusion technique with various concentrations of antibiotic disc on Mueller-Hinton agar plates (Bauer et al., 1966). Reading of the Muller-Hinton plates was carried out following 24 h of incubation and results were interpreted according to the recommendations of the CLSI (2005) (CIOMS, 1993).

Data Analysis

Data was analysed for descriptive statistics and presented in the form of tables. The results were interpreted in terms of frequencies and percentages

Ethical Consideration

Ethical approval of the study was sought from the University of Limpopo Institutional Research and Ethics Committee. Permission to collect samples was given by the Provincial Department of Health and National Health Laboratory Services (NHLS).

RESULTS

The various bacteria isolated from wound culture are shown in Table 1. The results indicate high cases of Staphylococci

species, especially *S. aureus* 25(29%) as compared to *S. epidermidis* 13(15%) and *S. saprophyticus* 1(1%). The trends decreased from lactose fermenting coliforms 13(15%), *Pseudomonas spp.* 10(11%), *Klebsiella spp.* 6(7%) and *E. coli* 3(3%) as well as streptococcus group D 3(3%). Fourteen specimens (16%) failed to grow on agar and might be due to an effective treatment regimen. Polymicrobial or mixed infections were not observed from the cultures.

Table 1 :

The various species of bacteria isolated from wound culture.

Bacterial isolate	No. of isolates	Percentage (%)
<i>S. aureus</i>	25	29
<i>S. epidermidis</i>	13	15
<i>S. saprophyticus</i>	1	1
<i>Klebsiella species</i>	6	7
<i>E. coli</i>	3	3
<i>Pseudomonas species</i>	10	11
Streptococcus Group D	3	3
Lactose fermenting coliforms	13	15
No growth	14	16

Table 2 :

Overall rates of antibiotic resistance (%) of Gram-positive bacteria.

Antibiotics	<i>S. aureus</i> n=25	<i>S. epidermidis</i> n=13	Streptococcus group D n=13
Linezolid (30µg)	8	8	0
Mupirocin (5µg)	16	38	67
Oxacillin (1µg)	24	39	100
Synergid (15µg)	4	8	8
Teicoplanin (30µg)	4	31	0
Vancomycin (30µg)	4	8	0

The antimicrobial susceptibility profiles of Gram-positive bacterial pathogens are shown in Table 2. As indicated in the table, *Staphylococcus aureus* exhibits high resistance to mupirocin (16%) and oxacillin (24%) while there is lowered resistance to linezolid (8%) and 4% to synergid, teicoplanin and vancomycin. *Staphylococcus epidermidis* on the other hand shows high resistance to mupirocin (38%), oxacillin (39%) and teicoplanin (31%). There is reduced resistance to linezolid, synergid and vancomycin at 8%. Streptococcus group D is totally resistant to oxacillin (100%) and also totally sensitive to linezolid, teicoplanin and vancomycin. There was only one *Staphylococcus saprophyticus* positive sample, which insignificantly exhibited sensitivity to all screened antibiotics (results not shown).

The susceptibility patterns of Gram-negative bacterial pathogens are shown in Table 3. *E. coli* isolates are totally resistant to ampicillin, tetracycline, cephalothin, cotrimoxazole and sulphatriad. The bacteria is also totally sensitive to colistin sulphate and gentamycin. Half of the *E.coli* isolates are resistant to streptomycin (50%). *Klebsiella* is totally resistant to cephalotin with reduced resistant to ampicillin (90%), sulphatriad (67%), streptomycin (50%), gentamycin (50%) tetracycline and cotrimoxazole at 33% as well as colistin sulphate at 17%. *Pseudomonas* isolates are totally resistant to cephalotin, with variable resistance to tetracycline (70%), sulphatriad and colistin sulphate at 60% as

well as cotrimoxazole (46%), ampicillin (40%), streptomycin (30%) and gentamycin (20%). The lactose fermenting coliforms are fairly resistant to ampicillin (77%), sulphatriad (54%), cotrimoxazole (46%), streptomycin (38%), tetracycline (31%), cephalotin (26%), colistin sulphate (23%) and gentamycin (8%)

Table 3

Overall rates of antibiotic resistance (%) of Gram-negative bacteria

Antibiotics	E. coli	Klebsiella spp.	Pseud spp.	Lactose fermenting Coliforms
	n=3	n=6	n=10	n=13
Ampicillin (10µg)	100	90	40	77
Tetracycline (25µg)	100	33	70	31
Colistin sulphate (25µg)	0	17	60	23
Cephalothin (5µg)	100	100	100	26
Cotrimoxazole (25µg)	100	33	40	46
Gentamycin (10µg)	0	50	20	8
Streptomycin (10µg)	50	50	30	38
Sulphatriad (200µg)	100	67	60	54

DISCUSSION

Wound infections have been a major concern among health care practitioners not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost-effective management within the health care system.

Our results attest to previous findings that *Staphylococcus aureus* (29%) remains the most reported pathogen playing a role in benign skin infections and life-threatening systemic clinical conditions (Marais et al., 2009; Mohammed et al., 2018), followed by coagulase negative Staphylococci (CoNS), *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* at 15% and 1%, respectively. The observation is similar to reports in, Nigeria, India, Thailand, and Japan (Gautam et al., 2013; Mama et al., 2014; Meseret et al., 2014; Pondei et al., 2013; Trojan et al., 2016; Rai et al., 2017). A different spectrum was reported by Manikandan and Asmath (2013) with *Pseudomonas aeruginosa* (43%) as the most common followed by *Staphylococcus aureus* (24.3%) (Manikandan et al., 2013; Rijal et al., 2017). *Pseudomonas aeruginosa* and *S. aureus* have been implicated in wound sepsis by a number of researchers (Manikandan et al., 2013; Pondei et al., 2013; Trojan et al., 2016). Methicillin-resistant *Staphylococcus aureus* (MRSA) as identified by resistance to oxacillin and vancomycin in this study is a serious global pathogen and economic-wise, negatively affects national health care systems (Heysell et al., 2011; Manikandan et al., 2013).

The role played by lactose fermenting coliforms and nosocomial pathogens like *Pseudomonas* and *Klebsiella*

species respectively should not be ignored. Of the 88 samples collected in our study the gram-negative bacilli made up of *Pseudomonas* (11%), *Klebsiella* (7%) and *Escherichia coli* (3%) species were isolated. These organisms have been reported as common contaminants of wounds in several studies. Manikandan and Asmath (2013) isolated *Pseudomonas aeruginosa* (42.9%), *Escherichia coli* (5.7%) and *Klebsiella pneumoniae* (2.8%) out of 70 wound swabs (Manikandan and Asmath, 2013). Similarly, Aizza et al. (2007) reported from 100 samples a frequency of Gram negative at 50.5%: *E. coli* (13.6%), *Klebsiella* species (12.8%), *Pseudomonas aeruginosa* (5.5%) and other *Pseudomonas* species (8.3%) (Aizza et al., 2007). From 200 pus swabs Gautam et al. (2013) reported *Klebsiella pneumoniae* (8%), *Escherichia coli* (7.3%) and *Pseudomonas aeruginosa* (5.3%) (Gautam et al., 2013). Recently, Mama et al. (2014) reported *E. coli* (20%), *Klebsiella pneumoniae* (14%), *Pseudomonas aeruginosa* (11%) (Mama et al., 2014). Emine et al. (2011) reported a similar spectrum from 100 postoperative wound infections with 71.8% due to Gram-negative bacilli (Emine et al., 2011). In the current study *Staphylococcus aureus* species showed a moderate level of resistance to oxacillin (24%) followed by mupirocin (16%) As compared to other studies with comparable sample size, our study showed that both bacterial groups exhibited multidrug resistance with the exception of prevalence of monocrobial tenden (Aizza et al., 2007; Emine et al., 2011; Meseret et al., 2014). There was a general resistance to oxacillin, by *Staphylococcus aureus* (24%), which was not in agreement with the high levels of resistance reported for amoxicillin by Mama, 2014 and Sule and Olusanya, 2000 to be 100%. In this study *Pseudomonas aeruginosa* exhibited 100% resistance to cephalothin, and reduced resistance to gentamicin consistent with reports elsewhere (Sule and Olusanya, 2000; Mama et al., 2014).

Generally, inadequate antimicrobial treatment defined as ineffective treatment of infection is an important factor in emergence of antibiotic resistant bacteria. Factors that contribute to inadequate antimicrobial treatment of hospitalized patients include; the prior use of antibiotic, broad spectrum antibiotics, prolonged hospital stay and the presence of invasive medical devices. The spread of resistant microorganisms through overcrowding and inadequate infection control policies also plays a role (Braga et al., 2013; Alharbi and Zayed, 2014).

In this study a monomicrobial growth was reported, inconsistent with the findings by Shittu et al. (2002), which gave polymicrobial flora associated with *Staphylococcus aureus* and other microorganisms (Shittu et al., 2002). Our study showed that *S. aureus*, *P. aeruginosa* and other coliforms with variable drug susceptibility profiles, are the most common agents found in infected wounds in the Limpopo Province. A strong recommendation for future studies is to consider the location and type of wound and include investigation for anaerobic bacteria. There is need for ongoing microbiological evaluation and antimicrobial susceptibility determination so that appropriate chemotherapy can be prescribed.

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